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NASA GRANT # NAG2-623

"Investigation of Wheat Coleoptile Response to Phototropic Stimulations"

Final Report
covering the period
November 1, 1989 to January 31, 1993

N93-18608

Unclas

G3/51 0145587

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Date of Submission: January 31st., 1993

(NASA-CR-192157) INVESTIGATION OF
WHEAT COLEOPTILE RESPONSE TO
PHOTOTROPIC STIMULATIONS Final
Report, 1 Nov. 1989 - 31 Jan. 1993
(University City Science Center)
34 p

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Abstract

This report provides a summary of the preparations for, and the conduct and post-flight data analysis of, the Spacelab flight investigation FOTRAN, which flew on the IML-1 mission (STS-42) in January, 1992. The investigation was designed to provide data on the responses of wheat seedlings to various blue-light stimuli given while the plants were exposed to orbital microgravity conditions. Before the flight, a number of hypotheses were established which were to be tested by the data from the flight and parallel ground studies. A description of the experiment protocol developed for the mission is provided, and an account of the activities supported during preparations for and support of the flight experiment is given. Details of the methods used to reduce and analyze the data from the flight are outlined.

The results obtained from the analysis of the flight data and its supporting pre- and post-flight ground studies completed to date are provided. While more data analysis is planned, we have progressed to a stage where an assessment of the responses of the wheat seedlings to the experimental treatments can be made, and a judgement made of the success of the FOTRAN flight experiment.

The seedlings in flight showed an unexpectedly rapid development, reaching a given height approximately 10 hours earlier than expected from pre-flight studies. A similar precocious development was also seen in Oat seedlings also grown in the GPPF equipment on IML-1. We have investigated this phenomenon and conclude that the precocious development is a result of early germination and emergence, and not enhanced growth rates. Post-flight testing of the GPPF hardware revealed that it is not possible to account for the effect on the basis of GPPF temperature control inaccuracies. The enhanced germination rate must be a product of some spaceflight effect other than microgravity or launch stress.

The plants reacted to the planned photostimulations given. Clear, records of phototropic responses were obtained. For the majority of photostimuli given, the phototropic response at zero g was undistinguishable from ground controls. The exception was for the 6 and 9 second stimulations, to which the flight plants showed a significantly greater response compared with the 1g controls. The large enhancement of response seen to all photostimulations in pre-flight clinostat simulations was not seen in the flight plants. Circumnutation was seen in nearly 50% of the seedlings in flight - a reduction from the 90% figure at 1g, but more than sufficient to confirm (in a different species) the findings of the SL-1 HEFLEX experiment that circumnutation occurs without a requirement for gravitational force. Examples of induction of circumnutation following a phototropic stimulation are reported, but the enhancement in circumnutation amplitude commonly observed during ground experiments was not seen in flight plants. Autotropic straightening of phototropic curvatures developed during the flight experiment were noted and analyzed. This provides unequivocal evidence that autotropic response does not

require g force for its expression. Many of the wheat coleoptiles underwent a strong nastic response after being transferred to zero g. We demonstrate that this response is most marked in short (<10mm) coleoptiles, and that the tendency is much reduced or eliminated if the zero g transfer occurs at a more mature seedling stage.

We conclude that the IML-1 FOTRAN experiment can be considered successful in achieving its primary objective; in permitting the testing of pre-defined hypotheses; and in revealing unexpected phenomena effecting plant growth under space flight conditions. As is the case with most scientific investigations, new questions are raised; these suggest further testing possibilities, both on the ground and in orbital studies.

1.0 INTRODUCTION

In 1978, in response to an Announcement of Opportunity by NASA, we proposed an investigation of wheat coleoptile responses to unilateral blue light stimulations given at 0g. The investigation became known by its acronym FOTRAN. The experiment was to be carried out using the Gravitational Plant Physiology Facility (GPPF) that was to be developed by our group at Philadelphia under the supervision of NASA Ames Research Center (ARC). The GPPF flight hardware was delivered to ARC in 1986. This report covers activities in support of the FOTRAN flight experiment on the First International Microgravity Laboratory Mission (IML-1) between November 1st., 1989 and January 31st 1993. This period covers pre-flight preparations, support of the IML-1 flight and the post-flight testing and reduction and analysis of the data collected during the flight. The main emphasis in this report will be on the scientific results obtained from the flight experiment, and the extent to which the objectives of the experiment were met.

The experiment and the GPPF hardware were designed to provide data on the responses of Wheat coleoptiles to phototropic stimulations of varied duration given to seedlings being held under microgravity conditions. The seedling responses (measured as growth curvatures towards or away from the stimulus) were recorded during the flight as time-lapse video tape records made using infra-red illumination. The illumination used is known to be physiologically inactive. Seedlings to be used in the flight were to be planted both on the ground and in orbit, to provide a number of batches for use throughout the mission. After transfer to orbit, ground-planted seedlings were transferred by the crew to 1g GPPF rotors to orient growth until ready for experimentation at about 75 hours of age. Seedlings planted in orbit were also placed on the rotors for the duration of their early development. At the correct age the seedlings were transferred to the GPPF Recording and Stimulus chamber (REST) where the 0g portion of the experiment protocol was performed. This protocol collected data from the seedlings for 5 hours before a timed exposure to unilateral blue light was given, and plant responses were monitored for a further 7 hours after the stimulus to observe the resultant curvatures.

The objectives of the FOTRAN experiment, as formally defined before the flight, were "To determine the time course of plant seedling curvature induced by a phototropic stimulation in a microgravity environment". Scientifically, we were interested in a number of discrete physiological responses of the growing coleoptiles, and the effects of the microgravity environment in modifying these responses. The rhythmic growth movement, circumnutation, was once thought to have an obligatory requirement for gravitational force for its expression. The Spacelab 1 experiment, HEFLEX, had demonstrated continued, but diminished amplitude, circumnutation in Sunflower seedlings at 1g. We hoped to confirm this finding in an unrelated species (wheat) and also to observe the effects of phototropic stimulations on this movement. At 1g, circumnutation amplitude is enhanced dramatically following an effective phototropic stimulus, and is often seen to be *entrained* by the stimulus (synchronization of the normally random pattern of movements in a number of seedlings). We also hoped, by the use of a range of stimulus durations, to determine whether the complex dose-response relationships seen at 1g are altered by the lack of effective gravitational force in orbit. Finally, we were interested in a little-

understood phenomenon, sometimes referred to as "autotropism". This is a straightening of a curvature produced by a tropic response in a growing plant organ. Thus, at 1 g, a tropic curvature is often a *transient* effect - the initial tropic curvature being "corrected" over time, leading to a straightening of previously curved portions of the organ. It has been hypothesized that this straightening is a gravity-dependent phenomenon. We expected that the data from the FOTRAN experiment would provide information on whether gravitational force was a requirement for this straightening response. Before the flight we proposed four "null" hypotheses that the FOTRAN experiment would test. These were:

- a) Seedling curvature (following a photostimulus) proceeds at the same rate and in the same direction in microgravity as in unit gravity.
- b) The extent of seedling curvature is the same in microgravity as in unit gravity.
- c) The seedling curvature does not exhibit reversal (autotropism), or lead to oscillations (circumnutation).
- d) The phototropic dose-response relationship is the same in microgravity as in unit gravity.

It should be pointed out that our expectation before the flight was that these hypotheses would *not* be validated by the flight experiment results. The success of the IML-1 FOTRAN experiment can be gauged by the extent to which the formal objective of the experiment was met, and by our ability to confirm or refute these four hypotheses as a result of the data gathered during the flight.

2.0 METHODS

The following description assumes that the reader is familiar with the design and functions of the components of the Gravitational Plant Physiology Facility (GPPF)

2.1 Description of FOTRAN Scientific Protocol.

The FOTRAN experiment used 3 day old wheat coleoptiles (*Triticum aestivum* cv Broom) as experimental material. The seed were planted in batches of 4 cubes, each containing 6 seeds at intervals both on the ground before launch (4 batches) and in flight (2 batches) to provide experimental material for testing throughout the flight. Cube seed trays were packed with moist "Promix-A" at a moisture content of 78% by weight. All cube parts had been ethylene oxide sterilized and allowed to offgas for a minimum of 24 hrs before use. Soil trays for the prime and the first back-up set (launch scrub contingency planting) were packed with the soil mix on 1/17/92 starting at 10 am EST. All packing operations took place under a laminar flow hood and sterile techniques were used during the packing and cube tray cleaning operations.

(Because of the problems encountered during the wet simulation in which retarded germination had been traced in part to the effects of sterilizing the promix in the KSC autoclave, the soil mix itself was not re-sterilized, but used as supplied by the manufacturer.) As the soil trays were packed and cleaned they were placed in autoclave sterilized boxes to minimize evaporative losses. Eight packed soil trays were wrapped in sterile Saran wrap and packaged in two "ZipLoc" bags. These soil trays, destined to be used during inflight plantings, were stored in the refrigerator

until transferred to the MASI and PCOC. The remaining soil trays were retained in closed boxes within the hood until scheduled ground plantings. Soil tray packing and cleaning operations were completed by 8 pm 1/17/92. Ground plantings were performed starting at 18:45 EST on 1/19/92, according to a pre-defined schedule designed to provide seedlings of the correct age for use at mission elapsed times (MET) as specified for FOTRAN operations in the pre-launch mission timeline. The planting schedule included the planting of batches for the prime launch and two 24-hr scrub contingency possibilities. Planning for preparations for further contingency launches was in place, but the successful launch of STS-42 on the first day made this unnecessary. At each scheduled planting, four pre-packed soil trays were planted with 6 selected wheat seeds, cleaned and placed in the four FOTRAN cubes designated for that batch. The cubes were carefully cleaned, wrapped in aluminum foil to minimize moisture loss, and transferred to the KSC plant growth room, which was maintained at 22.5°C. On 1/21/92 the cube batches for the first launch were removed from the growth room and packed into the PCOC together with GTHRES cubes and the temperature recorder (ATR). The packed PCOC was handed over to KSC integration team at 17:22 to be loaded into the Middeck locker at late access. Planting operations for launch scrub contingencies continued as scheduled until the successful launch.

In orbit, at MET 0/06:24, the planted cubes were transferred to the GPPF Left Test Rotor (LTR), which provided a 1g field and temperature control (nominal 22.5 °C) to allow normal plant development before the start of the experimental portion of the protocol. Seed tray packages for the inflight plantings were stored until required in the Plant Holding Compartment (PHC). In sequence (as specified in the mission timeline) the four cubes of each batch were removed from the LTR and placed in the Recording and Stimulus chamber (REST). This unit has locations for four cubes, each with its own independently-programmable photostimulus source. Once a batch was loaded into the REST, the crew initiated an automatic experiment sequence controlled by the GPPF micro-processor. The sequence provided a 5 hour period of timelapse (one frame per 10 minutes) video records of the plants before providing a photostimulus to each cube. The duration of the photostimulus was varied to provide different stimuli to each cube according to the overall experiment design. After the completion of the photostimulus, the REST collected further time lapse records until the end of the experiment for that batch, nominally 7 hours later. At the end of each experimental run, the crew removed the used cubes from the REST unit, and took samples of the atmosphere within a selected cube from the batch for later analysis. The plants in some batches were also chemically fixed in the biorack glove box. This sequence of operations was then repeated for subsequent batches of seedlings. In flight plantings were made by the crew, using the pre-packed seed trays, to provide experimental material for the latter part of the mission. Two such plantings were made. From time to time the crew changed out the video tape cassettes in the two GPPF VTRs as they became full. A total of 33 30-minute Umatic tapes were used during the mission. Table I gives a summary of the timings of the FOTRAN operations as flown during the mission.

At the end of flight operations the FOTRAN equipment was stowed, with fresh (unfixed) cubes being placed in the PCOC in the middeck locker to allow early

Table I: As-flown experiment timings for the IML-1 FOTRAN Experiment. All timings are given relative to the STS-42 launch at 09:52 EST on January 22, 1992.

	BATCH 1	BATCH 2	BATCH 3	BATCH 4	BATCH 5	BATCH 6
PLANTING	L-63:09	L-49:54	L-37:59	L-25:19	2/12:50	3/17:21
TRS TO CULTURE ROTOR	0/06:27	0/06:27	0/06:27	0/06:27	2/12:50	3/17:21
TRANSFER TO REST	0/12:47	1/02:36	1/14:09	2/03:06	5/06:57	5/17:21
AGE AT REST TRANSFER	75:50	76:30	76:08	76:27	66:07	48:00
START OF PHOTOSTIM	0/17:47	1/07:36	1/19:09	2/06:06	5/11:53	6/16:30
LONGEST PHOTOSTIM (mm:ss)	16:39	8:21	00:09	33:18	00:09	33:18
REMOVE FROM REST	1/01:47	1/13:54	2/02:22	2/14:58	5/17:18	7/01:46
AGE REMOVED FROM REST	88:56	87:08	86:18	86:17	77:10	80:25

recovery after landing. The VTR cassettes were stored in the module overhead locker and recovered 24 hrs after landing.

2.2 DESCRIPTION OF EXPERIMENT SUPPORT ACTIVITIES

2.2.1 Preflight Preparations.

Activities in support of the FOTRAN experiment in the pre-flight period included the development of suitable procedures to be followed for the ground planting, the crew procedures for use in flight and for the collection of the data and fixed and fresh (living) plant material at the Dryden landing facility. We were also actively engaged in the development of the GPPF portion of the overall mission timeline, an iterative process. As each new revision of the timeline was produced by the timeline engineers at Marshall Space Flight Center, we checked the proposal for compatibility with the scientific requirements of the experiment, and also against the logistical constraints imposed by the GPPF storage and other accommodations. Each revision of the timeline required additional checks of and corrections to the current crew procedures.

Crew training activities occurred in parallel with these activities. Training consisted of classroom introductions to the scientific background of the FOTRAN experiment and to the GPPF hardware capabilities. Hands-on training in the use of the hardware and in the manipulations of the plant material during the planting and fixation processes were also given. Most of this practical training was provided to the crew at the Marshall Payload Crew Training Center (PCTC), where a full mock-up of the IML-1 mission spacelab module was configured. This mock-up contained a good fidelity training model of GPPF which had a full range of functionalities, including simulating the symptoms of, and the required corrective actions to be taken in the case of, equipment malfunction.

Support was also provided during the process of integrating the GPPF flight hardware into the IML-1 Spacelab payload. We participated in the testing of the equipment during this progressive integration process. During integration a number of items within the hardware had to be replaced, most notably the REST video camera and

the GPPF Control Unit computer board. During level III/II integration (November 29, 1990) a calibration of the REST photosensors was performed, and during the Mission Sequence Test (April 14-18 1991) a calibration of the GPPF temperature sensors and video balance adjustments were performed. Due to extensive use in ground tests and crew training, the specimen growth containers (CUBES) used in GPPF became worn, and it was decided to manufacture a complete new set for the flight. Because of the tight turn-round timeline to be followed in the event of a launch scrub, we also manufactured a partial set of backup cubes to be planted for such contingencies. Manufacture of these items was carried out under our supervision at Philadelphia.

In preparation for the IML-1 mission we developed a number of management aids to be used by our POCC support team during the mission. These included software to display graphically the status of the GPPF experiment and scheduled future events in real time on a PC, and a video tape usage monitoring program to predict tape usage in the baseline timeline operations and as a result of replanned activities. We developed and provided logistical models that enabled the POCC support team to monitor the location of every GPPF experiment item during the mission. This was a significant task since there were a total of 136 separate moveable hardware components, and 44 timed crew activities that resulted in relocation of components within GPPF. The entire team received training at Marshall as required to qualify them to support the experiment in the MSFC SOA. The science team participated in a number of flight simulation exercises to complete this team training process.

On January 2nd, 1992 the entire FOTRAN science team attended the KSC Hangar L "wet" simulation in which a full simulation of the preparation of biological material for the flight was performed. The simulation exercised all aspects of the pre-flight procedures, the laboratories at Hangar L and the equipment required to support the preparations for flight. It provided the only opportunity to demonstrate the efficacy of the planned pre-flight procedures using KSC-provided equipment in producing the required healthy plant specimens for the FOTRAN flight experiment. This simulation proved to be the most vitally important of all the NASA simulations for this mission, since the plants prepared during the simulation were severely effected by a reduced germination/growth rate which would have lead to an almost total loss of the FOTRAN flight science, had the condition not been recognized and corrected. There was just sufficient time between the end of the simulation and the start of the pre-flight preparations for us to plan and carry out a series of tests which revealed that the prime cause of the growth failure was an off-gassing effect from a new batch of Nalgene boxes used to store the plant cubes in the growth room before PCOC loading. A secondary contributing factor was an as yet unexplained effect related to the sterilization of the soil mix in the KSC autoclave. By altering the procedures to avoid the use of the new Nalgene boxes (wrapping the cubes in foil) and using non-autoclaved soil mix we were able to demonstrate to our satisfaction that we could produce the required healthy seedlings for flight.

2.2.2 Launch Preparations

Pre flight preparations commenced 1/14/92 with lab preparations, soil mix preparations and cube cleaning and sterilization. All cube soil trays were packed with soil mix on 1/17/92, and the first FOTRAN seed planting activity occurred at 18:30 1/19/92. Flight and launch scrub contingency planting continued following the pre-planned schedule until the successful launch of STS-42 on 1/22/92. PCOC and MASI loading and handover to KSC were completed smoothly and on schedule, providing a complete set of 16 ground-planted cubes in four batches. Both the PCOC and the MASI contained, in addition, soil trays packed with moist promix to support two inflight plantings by the crew members. The plants prepared for launch scrub contingencies were not, fortunately, required, but were measured at KSC at a standard age for comparison with the flight specimens.

Engineering staff from our laboratory and members of the ARC support team had meanwhile arrived at MSFC to prepare experiment monitoring equipment situated in the SOA for the mission. This equipment was in place and checked out by L-1 day.

2.2.3 Flight Support Activities.

After the launch, the PI team at KSC travelled to Huntsville to the MSFC Science Operations Area (SOA) to join other personnel to monitor flight operations. The progress of the FOTRAN experiment and the performance of the GPPF equipment was monitored at the SOA throughout the mission on a 2 shift, 24-hr coverage basis. During the flight the PI, ARC and POCC Cadre teams worked well together. The training received in the pre-flight sims was extremely valuable, but had not fully prepared us for the extensive replanning activities that were required. Unexpectedly rapid development of the seedlings grown on GPPF necessitated rescheduling of many inflight operations. Only an efficient, coordinated effort by the GPPF SOA shift teams and the POCC cadre members allowed this replanning to be as successful as it was. The replanning effort required the expertise and dedication of a large number of individuals, all of whom performed well. The support documentation, displays and software developed before the flight performed their intended functions well.

2.2.4 Post Flight Recovery Activities.

Members of the team left the SOA at Marshall after completion of significant GPPF activities, but before the end of mission operations in order to travel to the Dryden Landing Facility to accept the return of plant material, video data tapes etc. Handover of the PCOC and MASI (with the unfixed flight specimens) to the PI team occurred approximately 3 hours after landing. The laboratory facilities at Dryden were well-equipped and specimen documentation was carried out without problems. During this process the unfixed specimens from flight were examined, photographed, measured, weighed and fixed. Any non-germinating seeds were placed on agar plates to be cultured. The entire documentation process was completed on the day of landing. On the following day, handover of the module stowage items occurred. These included the flight video data tapes, and the in-flight fixed plants. Tape

duplication facilities were provided by the Dryden Photo Labs and a complete copy set of the flight tapes were made. During this process, it was discovered that the F1 and G1 tapes had not successfully recorded data for the first 3/4 of the tape. These tapes are the first used tapes from VTR-F and VTR-G respectively, and the data loss is irrecoverable. The data lost represents the all of the pre-stimulus, and most of the post stimulus data for FOTRAN batch 1. Three downlink video episodes were recorded for this group of plants (1 pre stim, 2 post stim), and the last two hours of the run can be obtained from the VTR tapes after the initial noise anomaly. Thus some limited information from FOTRAN batch 1 is available, but the loss of the complete video record does represent a significant loss of science data. Lab operations at Dryden ended on Feb 3rd., 1992.

2.2.5 Post Flight Tests.

After the flight we arranged to conduct additional testing using the GPPF flight hardware at our laboratory. The purpose of this testing was for two major purposes, both of which arose as a result of the unexpectedly rapid development of the seedlings (wheat and oats) during the IML mission. Firstly, we wished to investigate the performance of the temperature monitoring and control systems of GPPF, to exclude the possibility that the advanced development seen was a result of GPPF internal temperatures being higher than intended during the flight due to some malfunction of this system. Secondly, whatever the cause of the unexpected development, it resulted in some experimental runs in flight being performed with seedlings at a more advanced stage than had been used for the preflight control studies. Ground control tests were therefore required to determine what effect this might have on seedling responses. The ground control studies used a protocol that closely followed the inflight crew procedures, and provided equivalent data.

2.2.6 Data Reduction and Analysis Methods.

Gas samples taken from selected cubes by the crew at the completion of the FOTRAN experiment runs were analyzed by an independent laboratory at Michigan State University, Department of Horticulture. The samples were analyzed by gas chromatography for Ethylene, CO₂ and O₂ content.

The prime data for the experiment was in the form of time-lapse video images present on 24 of the 33 GPPF video tapes recorded during the flight. (Because of timeline and other constraints, some of the GPPF video tapes contained GTHRES data only). After cataloging the information present on each tape data reduction was performed with the aid of Jandel Scientific's "Java" Image Analysis Software, running on IBM PCs. In brief, the process consists of replaying the tape on a Sony VO5800 Umatic format VCR, and capturing the selected image on a PC frame grabber. The Java software has facilities to enable the operator to make various measurements of the image using a mouse to select image features. The major measurements made on the images include coleoptile length (following calibration of the software to a known length standard) and measurements of the angular position of the apical third of each coleoptile relative to a reference direction (nominal cube vertical). Following completion of the measurement of the angles of all seedlings in all frames of a selected cube, the data were read into a specially-developed program that performed

tabulation and certain transformations on the data. For the angle measurements, the following standard data manipulations were performed in addition to the tabulation of the raw data: Angle measurements were transformed to correspond to the phototropic convention that positive responses are towards the light source; Data were corrected for the individual plant's position immediately before the onset of the stimulus (thus measuring response as change from this pre-stimulus position); Time references were transformed from mission elapsed time (MET) to minutes relative to the time of stimulus onset; Data smoothing was carried out using running average techniques over a 5 data point range; Means, standard errors and other relevant statistics were tabulated for each treatment. The transformed data were saved to disk files and used by graph plotting software to produce summary graphs of the data.

3.0 RESULTS

STS-42 was launched on January 22nd at 9:52 EST, 52 minutes behind the originally scheduled time due to local weather concerns. GPPF was activated some 5.5 hours after launch and the FOTRAN cubes were placed on the Test Rotors at MET 0/06:27. GPPF operated for a total of 166 hours during the mission (from MET 0/05:35 to 7/03:15). During this time a total of 362 time lapse "frames" were recorded from the FOTRAN experiment. Approximately 59 frames were lost from the recorded data of batch 1 due to a tape problem. (The VTRs had been placed in standby mode at GPPF power up, and during the six hours before data recording started the tape was loosely positioned over the rotating drum of the machines. This caused abrasion of the tape and occlusion of the record heads on both tape machines, which gradually cleared during normal recording. This problem had not been seen in post flight tests which exactly replicated the flight procedure. It must be concluded that zero g effects on the tape mechanism contributed to the problem.) The problem recurred in mid mission when the VTRs were in standby mode in the period between the completion of the ground-planted runs and start of the flight-planted runs. In this case only VTR-G was effected, and no FOTRAN data was lost. In addition to the tape record, periodic downlink of GPPF video was provided and recorded on the ground. Three of these downlink episodes occurred during the same period as the VTR data loss, providing some data that enabled partial recovery of the Batch 1 data. A nearly complete record of GPPF "housekeeping" was obtained from the RAU data stream, with insignificant dropouts caused by satellite data link problems. The crew obtained the gas samples and in-flight plant fixations as required.

3.1 Germination and seedling growth.

Following the experience during the wet simulation immediately pre-flight, when severely retarded growth had been seen (and the causes hopefully corrected), we were gratified to see that the wheat plants had germinated and grown well at the first opportunity to view the plants on downlink at MET 0/17:17. We did, however, note that the plants seemed somewhat taller than expected at this stage. The downlink opportunities were limited, and it was well into the second day that we realized that this "precocious growth" pattern was being repeated in more than the first FOTRAN batch and also by the oat seedlings used in the GTHRES experiment. It also appeared that the effect was increasingly apparent in each successive seedling batch

Table II - Wheat Germination Rate Summary. Total germination includes all seed that produced visible coleoptiles; "useable" percentage records only coleoptiles long enough for experimental analysis (longer than 10mm at the time of transfer to the REST).

FOTRAN - FLIGHT DATA	GERMINATION RATES	
	TOTAL %	USEABLE %
BATCH 1	95.8	83.3
BATCH 2	91.6	87.5
BATCH 3	100	95.8
BATCH 4	87.5	79.2
BATCH 5	75	66.6
BATCH 6	66.6	58.3
PLANTED PRE-FLIGHT	93.7	86.4
PLANTED IN FLIGHT	70.8	62.5
OVERALL GERMINATION	86.1	78.5

we saw, and that we were in danger of losing data in the later batches because the seedlings would become too tall to be experimentally useful if the trend continued. The mission timeline activities were rescheduled during the mission to correct for the advanced development. Germination rates for the wheat seeds were as shown in Table II. Total germination percentage reports all germinating seedlings, while "useable" seedlings are defined as those that were 10 mm or taller at the time of transfer to the REST unit (at about 75 hrs old). Germination of the ground-planted seeds was as expected from pre-flight tests, providing in excess of 80% of useable coleoptiles. The germination rate of the seeds planted in flight was somewhat depressed, but still yielded sufficient plants for scientific purposes. The reasons for the reduced germination of the flight-planted seed cannot be determined from the data.

As mentioned above, there was a clear tendency for later batches of seedlings to have taller than expected coleoptiles during the experimental period. To gain insight into the mechanism(s) that produced this effect, we measured the length of the coleoptiles in all FOTRAN batches at from the video record. Images were measured at intervals throughout the growth.

The data in figure 1 show the means and regression line of the growth of each batch of flight seedlings. The graph shows that the growth rates (slope) of the batches are similar. Statistical analysis shows that there is no significant difference between the slopes of any of the batches. This indicates that an increased growth rate was not the reason for the taller than expected wheat coleoptiles observed during the flight. If the regression lines are extrapolated to the age axis, an estimate of the time of emergence of the seedlings above the soil surface is obtained. This estimate is, of necessity, approximate, since the typical coleoptile growth curve is sigmoidal, rather than linear. However, the errors introduced by the linear approximation will be a

Figure 1: Extension growth as a function of seedling age for the FOTRAN wheat coleoptiles in flight. Inset: relationship between emergence and the period spent in orbit.

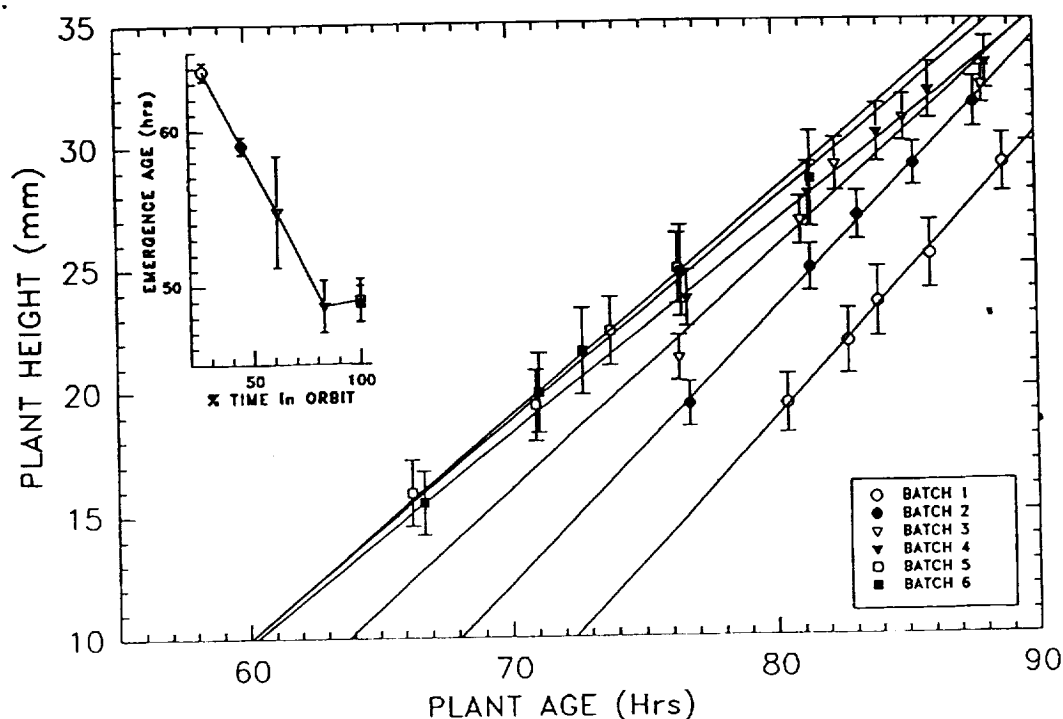


Table III: Growth Rates and Estimated Coleoptile Emergence Ages for the 6 flight batches.

IML-1 FLIGHT DATA	GROWTH RATE STATISTICS	
	GROWTH RATE (mm.hr ⁻¹)	COLEOPTILE EMERGENCE (hrs)
BATCH 1	1.10 ± 0.24	63.8 ± 0.86
BATCH 2	1.13 ± 0.11	59.1 ± 0.56
BATCH 3	0.92 ± 0.11	54.8 ± 3.57
BATCH 4	0.84 ± 0.13	48.7 ± 1.68
BATCH 5	0.91 ± 0.18	49.1 ± 1.39
BATCH 6	0.87 ± 0.14	48.9 ± 1.15

systematic overestimate of the age at emergence - all batches will be similarly effected. Table IV provides a comparison between the slopes and the age of emergence (extrapolated) for the 6 FOTRAN batches.

Comparisons between the age axis intercepts for the graphs show clearly that there is a systematic trend towards earlier seedling emergence from Batch 1 through 6. Thus the taller plants seem to be a product of enhanced development in the pre-emergence stages, the precocious development is a result of early germination and emergence, and not enhanced growth rates. Table V is a statistical matrix based on

t-test comparisons between the estimated times of emergence for the 6 batches

There is evidence that the greater the proportion of the pre-emergence stage that a wheat seedling is exposed to "spaceflight influences" the earlier will be its emergence above the soil, and therefore the taller the coleoptiles will be at any given age. The nature of the spaceflight effect that causes this effect is not known. It can be unequivocally stated that the influence is *not* one of microgravity. The FOTRAN batches were grown at 1g, either on a 1g rotor or a combination of earth gravity and

Table IV: Matrix showing the statistical significance of the differences between the age of emergence estimates for the 6 flight batches. The table shows the probability of finding differences as great as those observed by chance.

	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Batch 1	<0.001 ***	<0.05 **	<0.001 ***	<0.001 ***	<0.001 ***
Batch 2		>0.50 NS	<0.001 ***	<0.001 ***	<0.001 ***
Batch 3			>0.20 NS	>0.10 NS	>0.10 NS
Batch 4				>0.50 NS	>0.50 NS
Batch 5					>0.50 NS

a rotor. The less effected batches (1 through 4) all experienced approximately 5 hrs of microgravity between launch and GPPF activation. The batches with the most enhanced development were batches 5 and 6, which spent the entire pre-emergence period on a 1g rotor. By similar reasoning the effect cannot be a result of any stresses imposed by the launch (Batches 5 & 6 were launched as dry seed). The influence responsible for the precocious development must be some other environmental factor present in the orbital vehicle. In our early thinking about the effect and its possible causes, we considered that temperature might be a likely candidate. If the GPPF temperature control system were out of calibration, an elevated temperature of sufficient magnitude inside the culture rotors might produce the observed enhancement in development. After a series of extensive tests on the GPPF hardware run at Philadelphia (and reported elsewhere) we have come to the following conclusions. 1) To produce the magnitude of the observed effect would require that the GPPF culture rotors were well in excess of their nominal temperature setting during the mission (a minimum of 27°C, some 4-5° higher than any reported by the GPPF RAU data during the mission.) 2) Although the GPPF temperature system was found to be somewhat out of calibration (Data gathered during the Post-Flight Tests suggest that the Culture Rotor was between 0.7 and 1.6° C above the temperature reported by the downlinked data) this is not sufficient to account for the observed enhanced development. In the absence of any means on

the ground of testing other possible spaceflight environmental effects, we are unable to make any definitive determination of the cause(s) of the precocious development recorded from both wheat and oat seedlings on IML-1, but can only note the conclusion that the predominant influence is on the early (pre-emergence) stages of growth, and that the elongation growth rate of wheat is unaffected.

3.2 Phototropic responses

During the flight, wheat plants were exposed to a range of photostimulus durations from 3 seconds to 33 min 18 seconds. This range covers the first positive phototropic response through an indifferent range (where the photostimulus provokes \approx zero response) to the second positive response. Since it was desired to determine the effects of an effective stimulus on nutation, autotropism etc., the stimulus durations that (at 1 g) produced a marked response were more heavily replicated than those within the indifferent region. A summary of the stimuli given during the flight experiment are given in Table 6.

Table V: Details of Photostimuli given during the IML-1 FOTRAN experiment

		REST STAGE			
		1	2	3	4
STIMULUS EPISODE	1	3 00:03	9 00:09	99 01:39	999 16:39
	2	9 00:09	501 08:21	6 00:06	0 00:00
	3	6 00:06	9 00:09	0 00:00	3 00:03
	4	1998 33:18	3 00:03	999 16:39	6 00:06
	5	3 00:03	9 00:09	6 00:06	3 00:03
	6	3 00:03	1998 33:18	501 08:21	9 00:09

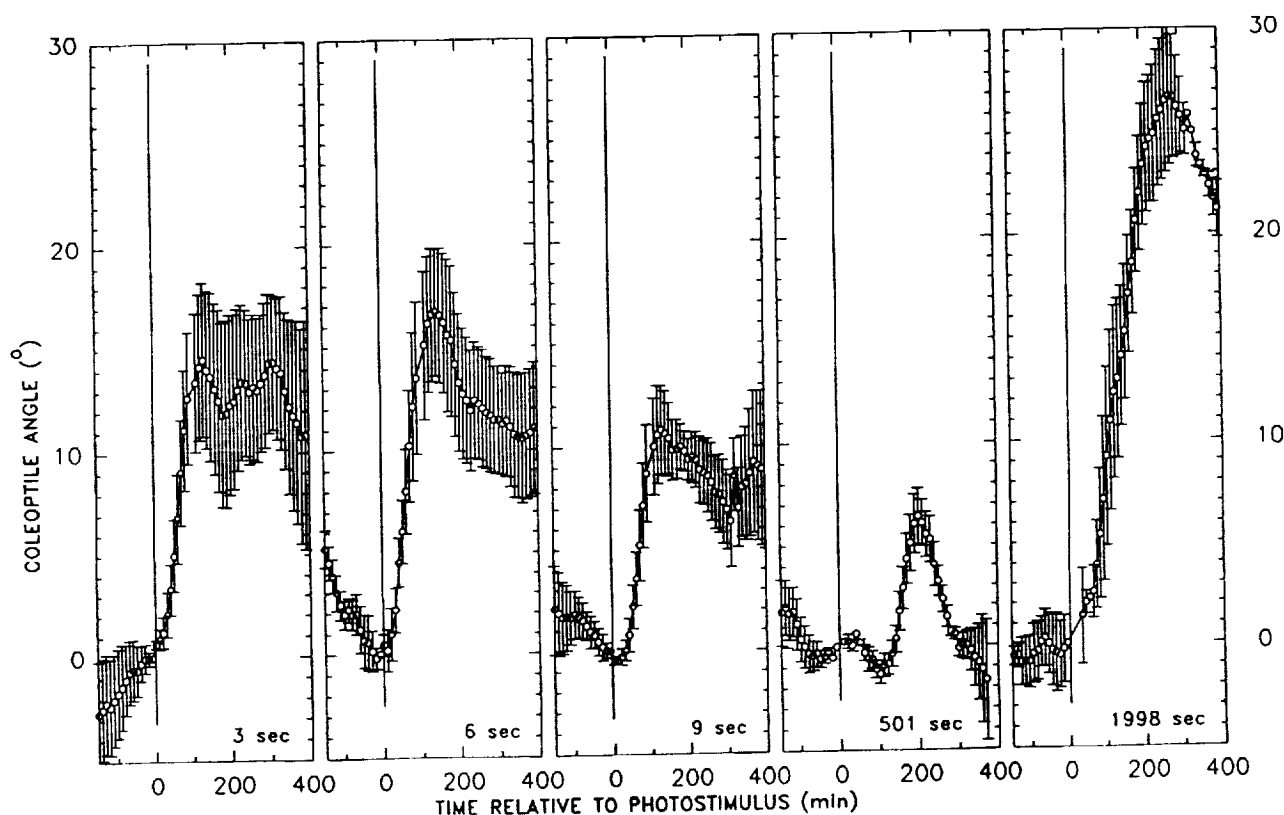
Analysis of the photoresponse parameters is still in progress. Interpretation of the data collected has been complicated by a number of factors. The precocious development observed in some batches of the flight seedlings, the presence of nastic curvatures that specially effected shorter coleoptiles (see later section) and the presence of apparently random curvatures in some seedlings all contributed to make the analyses more complex than had been expected pre-flight. The following account gives a report of the analyses to date.

Clear photoresponses were seen to all *effective* stimulations (i.e. those that produce a marked response at 1g). As expected, stimulus durations of 99 or 501 seconds did not produce seedling curvatures (these produce doses that fall within the indifferent

response range). There are a number of ways in which the extent of a photoresponse can be measured; the angular response produced at a standard time after the stimulus, the maximum response achieved, the maximum rate of curvature, etc. Thus far in our analysis of the IML-1 data we have concentrated on a standard measurement - the response at 100 minutes post-stimulus. Future analyses will consider other measures of phototropic response.

The FOTRAN protocol included in-flight controls, consisting of zero duration stimuli. These data were analyzed in the same way as the other data sets to provide a measure of the plant response when no photostimulus is given. When the response data to "real" photostimuli is compared with the data from these zero duration

Figure 2: Examples of time courses of photoresponses observed at 0 g during the IML-1 mission. Photostimuli of the indicated duration were given at time 0.

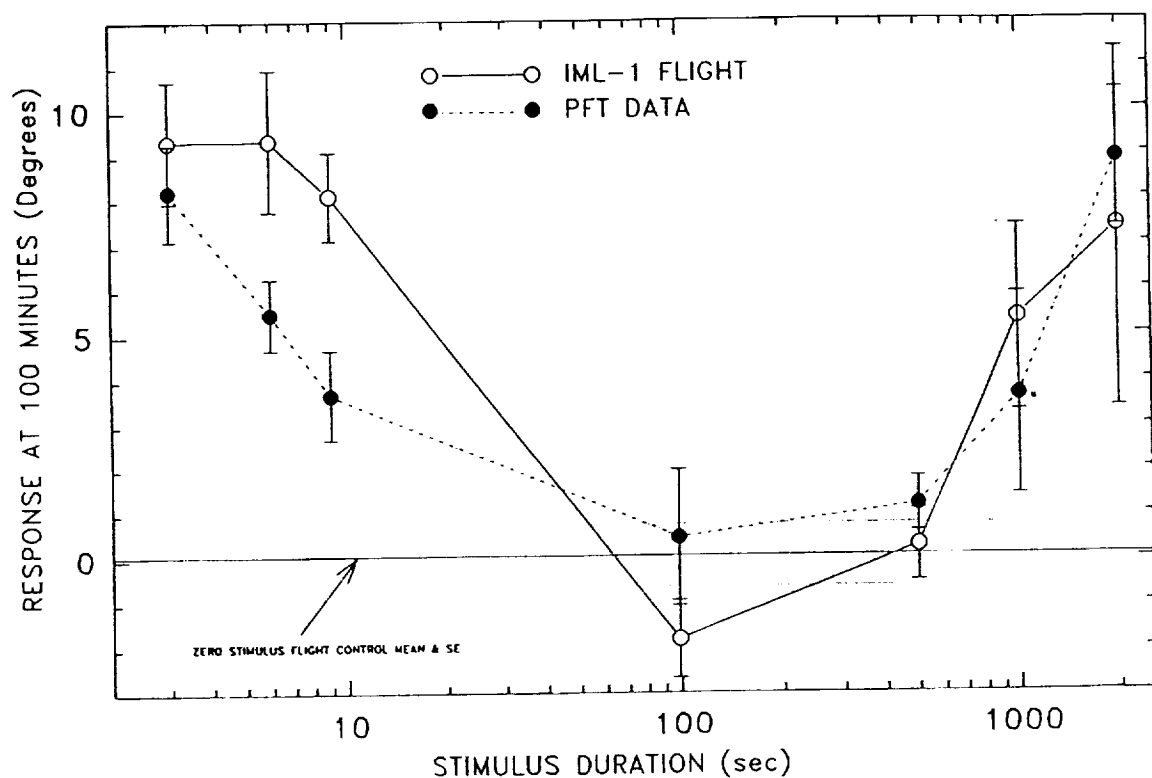


controls, it is found that the data from the 3, 6, 9 and 999 second stimuli are significantly different from the zero stimulus data, whereas the data from 99, 501 and 1998 seconds do not show statistically significant differences. In the case of the 1998 second data, this is at least in part due to the high variability of the data - caused by a bimodal response pattern, some plants showing large responses, others no response at all. It should be noted that the indifferent region treatments (99 and 501 seconds) do not normally cause a response at 1g, and therefore the lack of significant

differences for these treatments is not unexpected.

Figure 2 provides examples of photoresponses at 0g. There is variability between plants receiving the same photostimulus. Some plants show marked phototropic responses, while others, in the same batch, may show little or no response. This variability is regularly seen in plants at 1 g also, and is not a function of zero g exposure. The examples in Figure 2 were chosen to show the pattern of the phototropic response in plants which underwent an unequivocal reaction. We have not detected any apparent differences in the time courses of the responses occurring at 0 g. Responses, seen as curvatures towards the light source, are initiated within 10 - 20 minutes of the stimulus, and reach a maximum curvature approximately 100 minutes after the onset of the treatment. This pattern of response is similar to that found at 1g. We plan to undertake a detailed statistical investigation to compare flight with ground control response patterns during our future investigations of the IML-1 data to see if there are any subtle differences between the responses seen in flight and ground controls.

Figure 3: Dose response curves for data collected during the IML-1 mission and in post flight testing at 1 g.

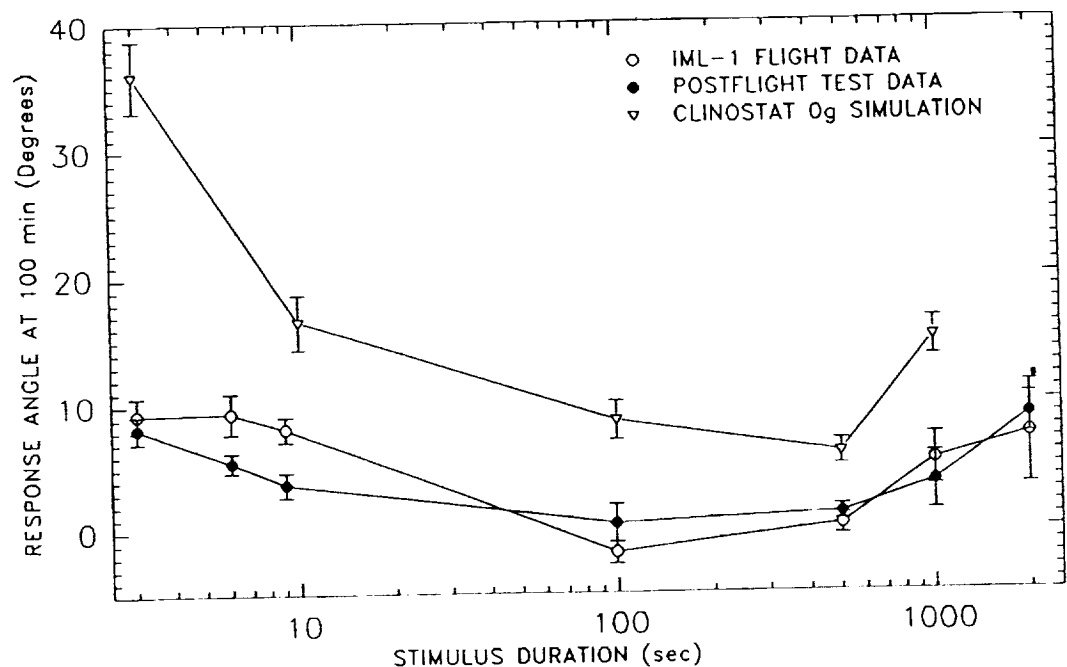


Under normal gravitational conditions, the relationship between the extent of the phototropic response and the stimulus dose (measured as *light intensity x stimulus duration*) is unexpectedly complex. In a large number of species investigated (including the FOTRAN subject, wheat) increasing the stimulus dose from zero does not lead to a progressive increase in the response magnitude. Typically a maximum response is seen at low dose levels, followed by a substantial medium-dose range in which the response elicited is small, or even negative (a curvature away from the light

direction). Following this indifferent dose range (at higher dose levels) marked positive curvatures are again produced. An objective of the FOTRAN experiment was to determine if this dose response relationship at 0g differed from that seen at 1 g. Ground studies using a clinostat had shown that the magnitude of the responses was considerably greater under *simulated* microgravity (Heathcote & Bircher 1987), so there was some expectation that larger responses might also occur in orbit.

The data shown in Figure 3 give comparisons between the IML-1 flight responses and a ground control data set collected during post flight tests (PFT) in the flight hardware after the return of IML-1. The only treatments which show statistically significant differences between 0g and 1g plants are 6 seconds and 9 seconds. In both these treatments the flight response is greater than the response developed on the ground. Figure 4 compares the flight and post-flight control data with the earlier clinostat results. Direct statistical comparison between the clinostat data and the flight and post flight sets has not been completed, but it is immediately apparent that the clinostat responses are markedly greater than either the flight or PFT. Once again the evidence is clear that the clinostat does not provide a reliable simulation of true microgravity effects. The flight data set is more closely similar to the 1 g control than to the clinostat simulation.

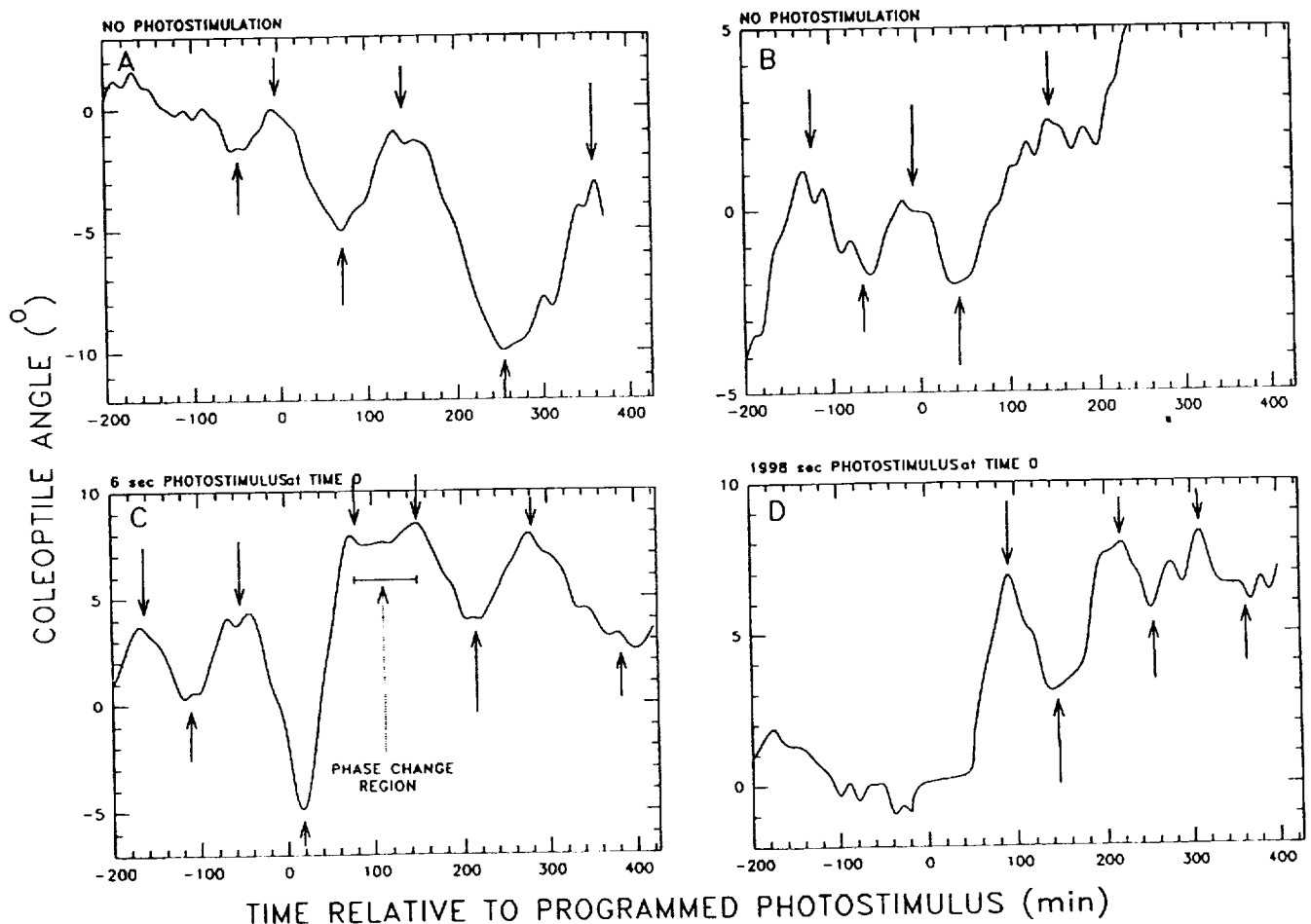
Figure 4: Dose response comparison between Flight, Post-Flight Tests at 1g, and Pre-Flight Clinostat 0g simulations



3.3 Circumnutation.

One of the objectives of the FOTRAN experiment was to determine if the rhythmic growth movement, circumnutation, occurs under microgravity conditions, and to determine the effects of phototropic stimulations on the rhythm. At 1g, phototropic responses often lead to the triggering of large amplitude circumnutations, or bring about increased amplitudes in pre-existing circumnutations. In wheat coleoptiles at 1 g circumnutation occurs in a high proportion (>80%) of unstimulated seedlings. The IML-1 FOTRAN data therefore provided an opportunity to confirm the findings of a previous Spacelab experiment (HEFLEX) which investigated nutation in Sunflower seedlings in the absence of gravity. This previous study found that circumnutations did persist under microgravity conditions, but with altered period length and amplitude, and a reduced frequency of occurrence compared with ground controls.

Figure 5. Circumnutation under microgravity conditions. The seedlings were transferred to 0 g at time 0, having been grown at 1g on the culture rotors. See text for details



As mentioned previously, the FOTRAN design included a number of observations of plants that were not subjected to any photostimulus - the in-flight controls. These observations provided approximately 12 hours of time lapse records for each seedling.

Since the average period length of circumnutation in wheat at 1 g is about 2.5 hours, this should allow sufficient time to detect such periodicities in the data (4 - 5 cycles). Theoretical models of circumnutation which postulate a requirement for gravitational force would predict that any circumnutation pre-existing in the seedlings (growing at 1 g on the culture rotor) would damp out after no more than 1-2 cycles after transfer to microgravity. The data from IML-1 provided several cases of persistent rhythmic activity in plants which were not photostimulated.

Examples of the time course of such circumnutations are given in Figure 5a and b. Circumnutations were also regularly observed in seedlings that did receive effective photostimulations, and in these cases the interaction between the two movements can be seen. Figure 5c shows a case where the photostimulus interacted with an established circumnutation rhythm, leading to phase changes in the rhythm. At 1g, there is a tendency for phototropic stimuli to enhance the amplitude of circumnutations and to produce synchronization of the plants within an experimental batch by means of phase shift phenomena. This effect is somewhat dependent on the timing of the light pulse relative to the phase of the circumnutation. We have not completed our analysis of the flight data in relation amplitude enhancement, but preliminary consideration of the data suggests that the augmentation seen in 1g controls is *not* repeated in the flight specimens. This would be in line with the concept that the augmentation at 1 g is a result of gravitropic amplification of the nutational movements. Figure 5d is an example of *initiation* of circumnutation activity apparently triggered by a phototropic stimulus. This indicates that, even in the absence of gravitropic amplification of circumnutation (at 0g), a phototropic stimulation can, under appropriate circumstances initiate a stable rhythmicity in a previously arrhythmic plant.

To provide an overall comparison between the characteristics of circumnutation

Table VI Summary of the circumnutation parameters found in the IML-1 FOTRAN experiment and a pre-flight ground study (IGS-1):

Circumnutation Parameter	0g (IML-1)	1g (IGS 1)
Plants showing clear rhythmicity	46%	91%
Period length (min)	120	140
Standard Error	± 4.5	± 3.7

occurring under orbital conditions with that observed at 1g, we determined period length for all clearly defined circumnutations seen in the flight data, and a control data set collected during the pre-flight period. We also determined the proportion of the total coleoptiles that showed clear circumnutational activity. These data are summarized in Table VII. There is a reduction in the proportion of seedlings that exhibit circumnutation at 0g, but there is no doubt that circumnutational activity is present in almost half of the flight seedlings. There is also a statistically-significant reduction in the mean period length of circumnutation in the flight seedlings as

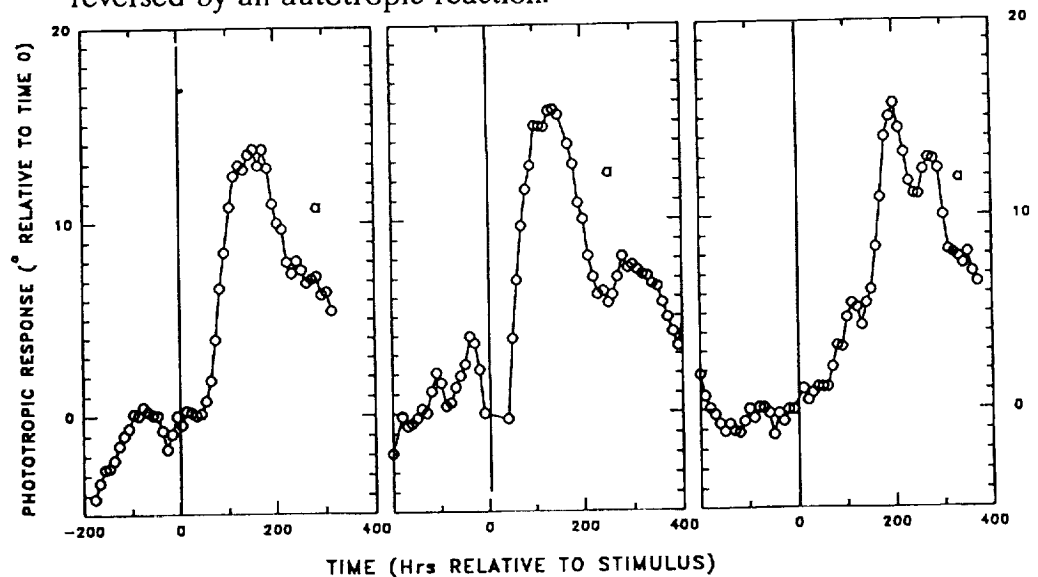
compared with the ground control plants. Similar effects on period length and the frequency of nutation were reported from the Spacelab 1 HEFLEX experiment. The FOTRAN data clearly support the contention derived from the HEFLEX data that the process of circumnutation is not *dependent* on the presence of the gravitational force, but that certain characteristics of the movement are *modified* by the presence of an effective g force.

3.4 Autotropism

The phenomenon of autotropism (a straightening reaction following a tropic or other curvature) is clearly demonstrated by the data from the flight plants. At 1g, it is difficult to be certain that any straightening of a plant organ observed following the induction of a tropistic curvature is not caused by gravitropic influences. When the observations are carried out in microgravity, however, as with the FOTRAN data, it is clear that if straightening is observed the process is *not* dependent on the gravitational force.

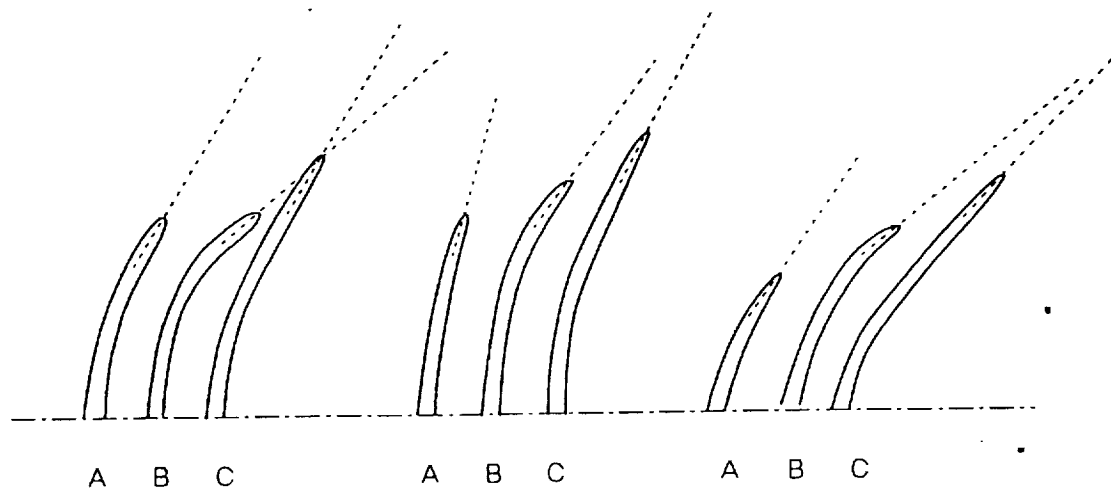
The data from the flight clearly show evidence of straightening of curvatures induced by the photostimuli. Indications of this can be seen from the time course graphs of

Figure 6 Typical examples of plant phototropic responses that are partially reversed by an autotropic reaction.



the plant responses, as for example in the typical examples shown in Figure 6. The initial curvatures develop to a maximum deviation at or about 100 minutes after the stimulus. The curvature then reduces over the next 3 - 4 hours, typically resulting in a plant angle at the end of the record that is approximately half of the magnitude of the maximum phototropic response curvature. Although the most likely explanation of this observation is that the phototropically-induced curvature is straightening, it would be theoretically possible to obtain the same effect if the plant developed a new, oppositely-directed curvature, for example at the base of the seedling. When the video data is examined, however, it can be seen that the angle reductions do, in fact, result from a straightening, "autotropic" reaction. There is clear evidence

Figure 7: Tracings of three images of coleoptiles shown A) before the phototropic stimulus, B) at maximum response, and C) after autotropic straightening (7 hours after the stimulus).



that the curvatures developed at maximum response (at 100 minutes after stimulation) are reduced in magnitude by the end of the experimental run - straightening reactions (autotropism) have occurred. Tracings of coleoptiles taken from the video images are given in Figure 7. Since the entire time during which the phototropic curvature was developed and, later, diminished, took place under microgravity conditions it must be concluded that the process of autotropic response does not have an obligatory requirement for g force.

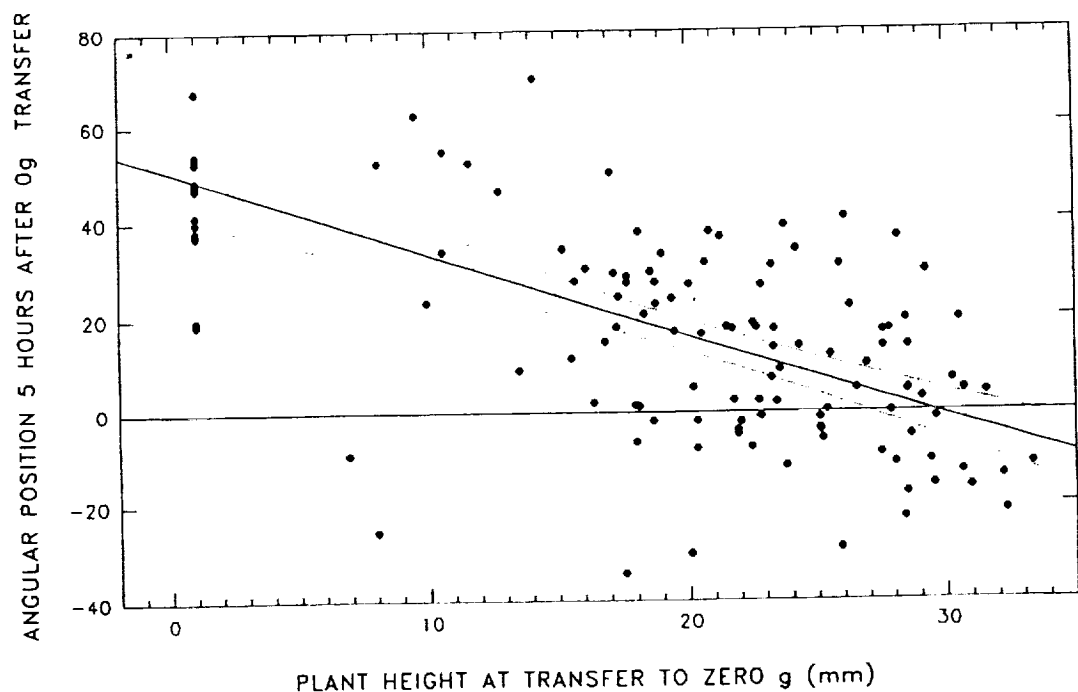
3.5 Nastic and other spontaneous movements.

The analysis of the phototropic and circumnutation data from the video tapes was complicated by the presence of unexpected spontaneous curvatures that occurred in a proportion of the flight seedlings. There was a marked tendency in some of the FOTRAN batches for seedlings to undergo a curvature following transfer to $0g$ in the REST. Visual observation of the video record revealed that the tendency appeared to be most marked in seedlings that were short ($<5-10$ mm) when transferred to the REST. Most commonly the curvatures occurred in the same direction - towards the photostimulus window. Since seed planting procedures used for the wheat seed ensured that all seed were planted such that the coleoptile developed on the side of the seed closest to the window, the observation is consistent with a strong nastic response of the coleoptile away from the seed. The curvatures, where present, appeared to be predominantly in the basal third of the coleoptile. The final curvatures led, on average, to a coleoptile axis inclined at about 40° to the cube vertical axis. In extreme (and rare) instances, the seedling became parallel with the soil surface. In most instances the nastic response appears to be induced by the transfer to $0g$, or some event associated with that transfer. Similar curvatures have been reported previously from seedlings on clinostats. In the literature relating to clinostat-induced nastic curvatures of cereal coleoptiles, the direction of the curvature is species-dependent, but always in the plane through the coleoptile longitudinal axis and at right angles to the longest axis of the elliptical cross section of the coleoptile.

The IML-1 flight data from wheat therefore appears to represent a similar phenomenon to that observed in clinostat experiments.

As mentioned above, the data seemed to indicate that short seedlings were much more susceptible to the nastic curvatures than more mature seedlings. This was strongly indicated by the data from Batch 6. These seedlings were intentionally transferred to the REST unit at an early stage of development (48hrs rather than the nominal 75 hrs). This was done to obtain data to characterize the precocious development of the seedlings which had been identified from the downlinked video images obtained early in the mission. We felt that data on the growth of the seedling from an earlier stage than normally available from FOTRAN data sets might be valuable in our investigation the anomalous development. All Batch 6 seedlings (which were pre-emergent at 48 hrs) developed a uniform curvature of around 40°. In other batches, seedlings that were shorter than normal were most likely to undergo similar magnitude curvatures, while taller coleoptiles were unaffected. To quantify this data, we measured the height of all seedlings at the time of transfer to 0g, and

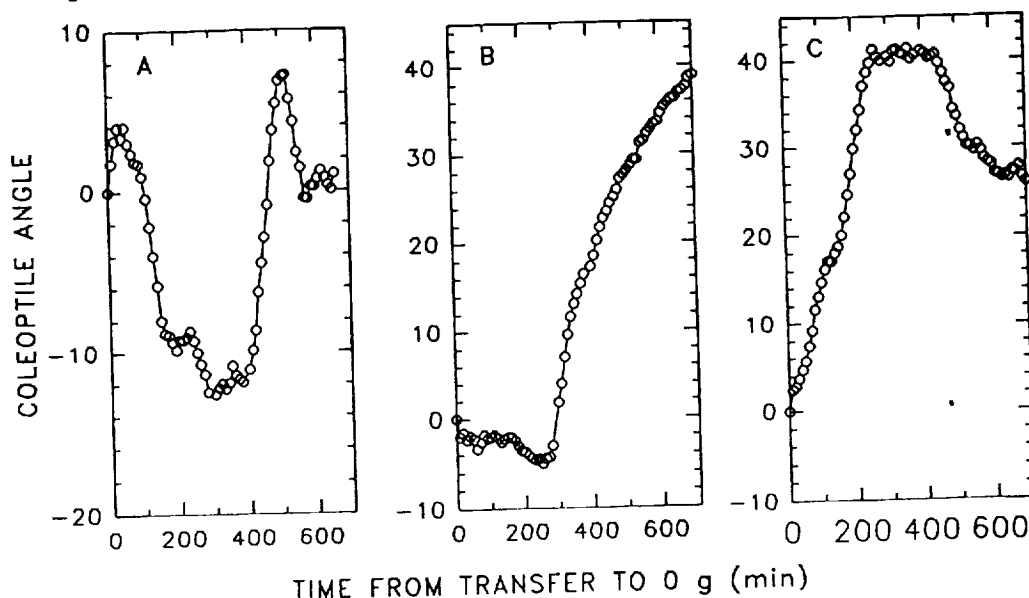
Figure 8: Scatter plot (with regression & 95% confidence limits) showing the relationship between coleoptile height at transfer to 0g and the degree of nastic curvature developed.



the angle relative to the cube vertical just before the photostimulus (after about 5 hours exposure to 0 g). These data are plotted as a scatter plot in Figure 8. The regression line through this scatter is statistically significant at <0.001 probability level, and the regression accounts for some 35% of the observed data variability. The statistical analysis confirms that nastic response curvatures are seen in the flight data -

the observed curvatures are predominantly in a positive direction, rather than randomly oriented, and that the degree of this nastic response is greatest in shorter coleoptiles.

Figure 9: Examples of apparently spontaneous curvatures in wheat coleoptiles at 0g. The plants in these examples were not subject to photostimulation.



More occasionally we observed curvatures that had a more random nature, both in timing of the onset of curvature, and in the direction of curvature. In some cases, the time course graphs show abrupt cessation of bending activity, followed later by either continued or reverse-direction curvature development. We believe that these "spontaneous" curvatures are different from the nastic curvatures described above, since they do not show a preferred direction, undergo directional reversals, and do not appear to be triggered by the transfer to zero g or by the photostimulus. Since these curvatures occurred infrequently, we can not come to any useful conclusion as to their nature or causation, but do note that some examples of apparently spontaneous and randomly-directed movements did occur during the growth of wheat coleoptiles at 0g and that in some cases the curvatures attained significant magnitude. The examples given in Figure 9 are taken from the Flight controls (zero duration photostimulus) and therefore cannot be a result of photostimulus. Similar examples of apparently random behavior are also seen in photostimulated coleoptiles, but in these cases the phototropic responses are superimposed, leading to difficulties in interpretation of the data. The plant shown in Figure 9A shows two, oppositely-directed spontaneous curvatures. The first, between 40 and about 200 minutes after the transfer to the REST, is a negative curvature of some 12°. (Note that this curvature is in the opposite direction to the positive nastic responses described earlier.) After a period of little activity of about 1 hour, a strong positive curvature of 18° occurs. Some of the curvature changes seen are probably due to circumnutation, but these seem to be superimposed on the spontaneous movements. Figure 9B shows a particularly striking example of the sudden onset of a very strong curvature. The plant is quiescent for some 4.5 hours, after which a pronounced

positively directed curvature occurs. This leads to a final curvature in excess of 40° before the end of the data. The coleoptile of Figure 9C shows a rapid, positive curvature that is apparent from the start of the data. After some 250 minutes of a more or less constant rate of curvature, the movement abruptly stops. A quiescent period about 2 hours long is followed by a strong reverse (negative) curvature that continues to the end of the record.

3.6 Ancillary Data

a) Gas sampling data

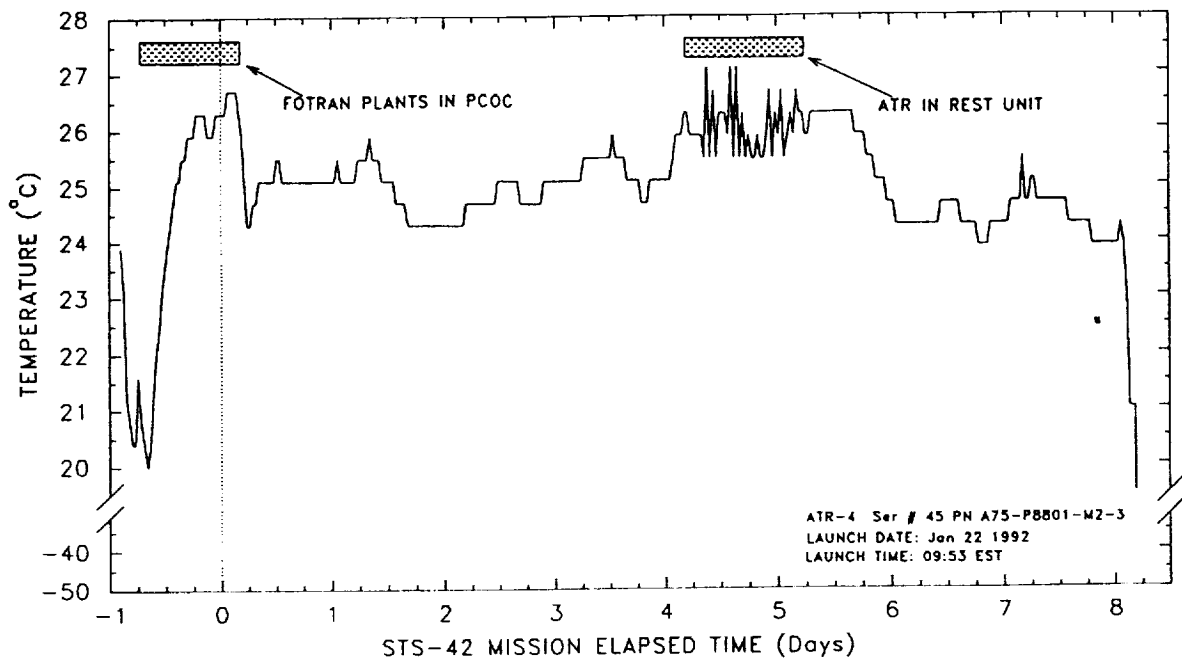
Table VII: Gas sample data from the GPPF plant cubes. The shaded data are from FOTRAN cubes. GTHRES data (unshaded) are included for completeness.

CUBE ID	MET OF SAMPLE	COMPONENT		
		O ₂ %	CO ₂ %	C ₂ H ₄ ppm
F1	1/01:51	21.6	0.2	0.02
F4	2/15:06	22.2	0.3	0.01
F6	1/13:54	19.9	0.2	0.01
F7	2/03:30	18.1	0.3	0.01
20	1/18:30	18.5	0.2	0.02
35	2/14:06	17.6	0.3	0.02
45	4/23:25	17.0	0.4	0.02

The crew took four gas samples from FOTRAN cubes at intervals throughout the mission. One sample was removed from a randomly selected cube at the end of each of the first four experimental runs. Similar samples were also removed from the GTHRES cubes. The gas samples, in sealed gas syringes were sent for analysis immediately following recovery of the module stowage (R+24hrs). Analyses were carried out on February 4th, 1992 for Ethene (Ethylene), Oxygen and Carbon dioxide. Table VII provides a summary of the data derived from all the GPPF gas samples. None of the levels found were outside the expected range, being similar to values found in cubes in previous ground studies. The levels are within acceptable physiological limits for the FOTRAN experiment.

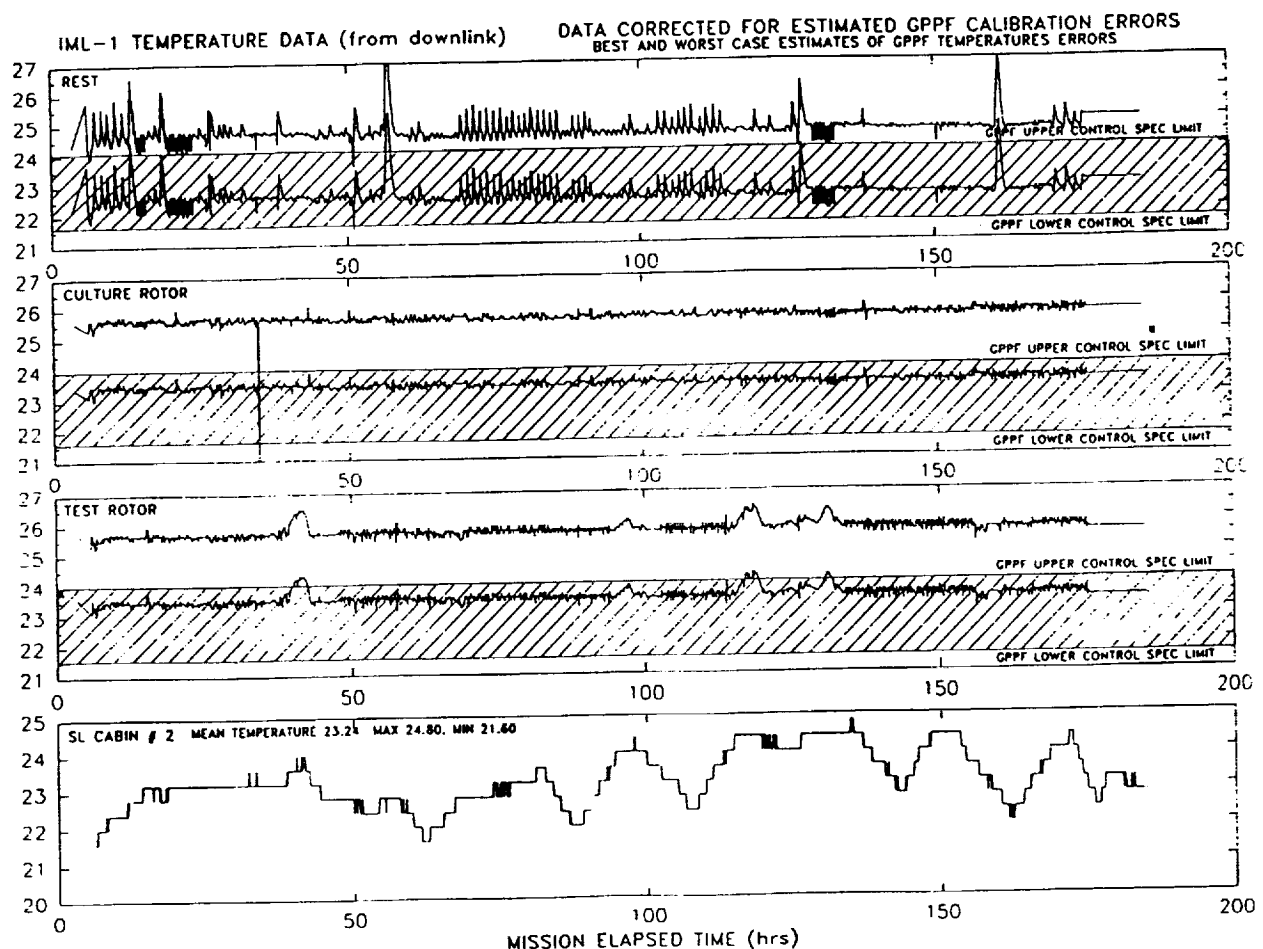
During the mission a number of environmental measurements were made of relevance to the FOTRAN experiment. These included the "housekeeping" data produced by the GPPF equipment, and other more general data, such as spacelab environmental temperatures etc. These were downlinked and recorded, together with many other Spacelab and Shuttle data, on the mission computers. We also gathered data from the ATR (Automatic Temperature Recorder) that was packed with the cubes in the PCOC to record Middeck temperatures within the PCOC during ascent. For a 5 hour period during the mission the ATR was taken from the PCOC and placed in the REST unit (vacant at the time) to provide an independent confirmation

Figure 10: Temperature level data from the PCOC interior in the middeck lockers and within the GPPF REST unit (shaded portion) during the IML-1 mission



of GPPF temperature. The data collected by the ATR unit was decoded and is provided as Figure 10. The reliability of the temperature data derived from the GPPF housekeeping data was investigated at length in the Post-flight tests of GPPF (reported elsewhere). There remains some uncertainty regarding the actual temperatures within GPPF during the flight. The calibration of the GPPF sensors was found to be slightly inaccurate during the post flight tests. These errors, together with an uncertainty regarding the temperature environment within the GPPF Control Unit (which could have influenced the accuracy of the sensor readout system), contribute to an overall temperature level uncertainty. The post flight tests allowed us to define outside limits for the possible temperature levels actually prevailing within each GPPF unit throughout the mission. These are presented in Figure 11 as a pair of lines for each GPPF unit, representing the upper and lower limit of the true temperature. The shaded range is the desired GPPF control set pre-mission.

Figure 11: GPPF unit temperatures during the IML-1 mission. The temperature levels are shown as worst case upper and lower limits for the actual unit temperatures based on uncertainties over the prevailing avionics temperature.



4.0 SUMMARY OF SIGNIFICANT ACCOMPLISHMENTS

As described in the introduction, a number of pre-flight objectives and working hypotheses were established before the mission. Success of the flight experiment can therefore be gauged by our ability to meet the objectives set before the flight, and the extent to which the stated hypotheses can be statistically tested with the data obtained from the experiment. The primary objective of the FOTRAN experiment was stated as: "...determine the time course of plant seedling curvature induced by a phototropic stimulation in a microgravity environment". The data returned from the IML-1 flight clearly met this requirement. Responses by individual plants to all normally effective photostimuli were seen and measured from the data and are shown to be statistically different from plants which received no photostimulation in flight.

Although the loss of an extensive part of the video recorded data from Batch 1 was disappointing, sufficient information was available from downlinked images to obtain some useful measures of response from Batch 1 plants. Moreover, the additional of Batch 6 following the mission extension compensated for the earlier loss. Sufficient data were obtained from individual plants to enable statistical assessments of mean response to the various photostimulations to be made and for comparisons to be

made between treatments. The experiment can therefore be judged a success on the basis that it achieved the originally defined experimental objective.

The experiment was also designed to allow the testing of 4 "null" hypotheses. In our analyses to date we have been able to provide definitive or partial answers to 3 of these four tests of hypothesis, and the data appears suitable to make the forth test, on which analytical work is still in progress. A summary of the progress on the four pre-flight hypotheses follows.

Hypothesis a): Seedling curvature (following a photostimulus) proceeds at the same rate and in the same direction in microgravity as in unit gravity.

The curvatures observed in the wheat seedlings in flight clearly follow the same positive direction (towards the light source) as is seen in all ground studies. Precise measurements of individual curvature rates have not been made, but the data will allow this assessment to be made in future analyses. Measurements of the seedling response at 100 minutes after the stimulus have been made, and are clearly related to the rate of curvature during this period. On the basis of the 100 minute parameter we find that for the majority of the phototropic stimulus durations used, that there is no significant difference between the curvatures developing under microgravity and those on the ground. This is however not the case for 6 and 9 second stimuli. In these cases the response in space is greater than the ground control at a statistically significant level. This would imply that, for these stimulus durations only, the above *hypothesis is not confirmed* by the data from the FOTRAN experiment. At all other stimulus durations the hypothesis is not questioned.

Hypothesis b): The extent of seedling curvature is the same in microgravity as in unit gravity.

The final analysis of this question, which relates to the maximum curvature achieved, and the duration of the response phase, has not been completed. The data appear to be such that it will be possible to reach a conclusion on this question, despite the interactions between the phototropic response and other movement patterns seen in the seedlings in space.

Hypothesis c): The seedling curvature does not exhibit reversal (autotropism), or lead to oscillations (circumnutation).

In both cases (autotropism and circumnutation) the flight data show that this hypothesis is not valid. While not all seedlings exhibited either behavior, a sufficient number examples are present to state unequivocally that *autotropic reversal of curvature can occur in the absence of effective gravitational force*. Circumnutation was present in nearly half of the seedlings in flight. This is a reduction from the 90% proportion seen during ground studies. However, it is apparent that *the absence of gravity does not preclude expression of the rhythm in wheat seedlings*. Rhythmicities were also observed in those batches of seedlings that were not photostimulated. Examples of both phase shifting behavior in existing rhythms and initiation of circumnutation by the phototropic stimulations are seen in the data (see section 3.3). Thus, although

the effects are shown by relatively few individual plants, it can be stated that *phototropic stimulations can initiate rhythmicity, or modify, by phase shift, existing circumnutations in wheat coleoptiles at 0g.*

Hypothesis d): The phototropic dose-response relationship is the same in microgravity as in unit gravity.

The evidence presented in section 3.2 on the dose response curve of wheat phototropism show that for the majority of stimulus durations the mean response seen in zero g conditions is not statistically different from the mean response found in 1 g controls. For two stimulus durations - 6 and 9 seconds - the differences between the flight and ground data *are* statistically significant. In both cases the response is a larger angle in zero g. This same trend is seen in the 3 second treatment means, although the data do not reach a statistical level sufficient to conclude that the differences were not due to chance sampling error. *The hypothesis tested fails to be confirmed* in this case, since the 0g and 1g dose response curves are found to be different over the range between 6 and 9 second stimulus durations. While the data could be taken to be indicative of a shift in the phototropic System I responses towards higher doses at 0g, there were insufficient experimental data points available to substantiate this as a real effect.

It is very clear that the large increase in response magnitude reported previously from clinostat simulation experiments were not repeated in true microgravity. This was rather surprising, since an enhancement of the phototropic curvature could be expected theoretically, due to the absence of gravitropic counter-reactions in microgravity. Only the 6 and 9 second stimuli in flight produced greater responses (approximately 5°) than the 1g controls, but this was not as great as the 12° enhancement produced on the clinostat. No significant enhancement was shown at 3 seconds duration in flight, compared to the massive 25° increase for this stimulus on the clinostat. *Thus the clinostat clearly failed to simulate the effects on phototropic response patterns that were produced during the exposure to true orbital microgravity conditions.*

In addition to achieving the pre-flight objective of the experiment, and providing tests for a number of pre-flight hypotheses, the FOTRAN experiment also provided evidence of several unexpected or novel phenomena associated with the growth of wheat seedlings in the orbital laboratory.

The precocious development phenomenon was not predicted pre-flight. Measurements of the plants from the flight data tapes clearly demonstrate that the effect was due to an earlier emergence of the coleoptile from the seed, rather than an effect on the elongation growth of the coleoptiles. Our post-flight tests on the temperature control and monitoring system of the GPPF flight hardware failed to provide any evidence that would suggest that the temperatures inside the GPPF units were sufficient to explain the observed effects. The effect is real, it was unexpected, its physiological mechanism is not understood, and it may be of significance for future planning of spaceflight experiments using germinating plants. At this stage we can not predict how common this effect will prove to be -certainly we know of no other reports of enhancement of germination from space flight experiments. The effect was not

confined to the wheat plants, but also effected the oat seedlings used in the GTHRES experiment.

The development of strong nastic curvatures following the transfer of young coleoptiles to microgravity was also unexpected. With hindsight, it could be related to similar phenomena reported from previous clinostat studies. These effects had largely been considered as clinostat artifacts. From the FOTRAN flight data we were able to confirm statistically that nastic curvatures developed most strongly in coleoptiles that were short (physiologically young) when transferred to microgravity conditions. Coleoptiles raised at 1g (on the rotors) until they reached about 15mm long did not seem to be effected by the nastic response. This may be of significance for future experiments (or crop production projects) using cereal plants grown from seed in space. In the absence of gravitational force during germination and early growth, cereals may develop similar nastic curvatures which might lead to the development of inefficient photosynthetic canopies, for example. Although it may be the case that phototropically-directed growth will be produced in light-grown seedlings, there is no direct evidence from space flight experiments that support this possibility. Even if such phototropic counter-measures are not used, it would be possible to orient the seed axis during planting to produce a mature seedling of a desired orientation relative to the soil surface. However, the correct orientation for the seed of a particular cereal species would need to be determined by space experiment, since the magnitude and the direction of the nastic curvature (at least on the clinostat) is species-specific.

Apparently spontaneous and randomly-directed curvatures were observed in a relatively small number of seedlings. Although not common, these movements are of theoretical interest. The movements, described earlier in section 3.5, seem to stop and start without apparent triggering from external cues. Certainly the movements do not effect all seedlings within a batch of cube, and no case was observed in which several seedlings showed synchronization of the onset or cessation of these movements, which might be indicative of an outside cue. We know of no reports of similar effects in the literature on plants grown in microgravity.

To summarize: The IML-1 FOTRAN experiment can be considered successful in achieving its primary objective; in permitting the testing of pre-defined hypotheses; and in revealing unexpected phenomena effecting plant growth under space flight conditions. As is the case with most scientific investigations, new questions are raised; these suggest further testing possibilities, both on the ground and in orbital studies.

5.0 FOLLOW UP

In the period following the submission of this final report we will continue to refine the analysis of the IML-1 flight data and ground controls. This will include performing additional analyses of phototropic response using different parameters, such a maximum response, curvature rates, etc. Measurement of response lag time, maximum curvature rates and timing, maximum overall response time etc will be obtained from the reduced data files, and used to investigate the possibility of different phototropic response characteristics between the flight and ground controls.

We also plan to investigate the feasibility of modifying the method of data reduction using the Java image analysis software to separate the effects of phototropic responses from nastic curvatures occurring in the same coleoptile.

We will continue with the preparation of papers reporting the results of the FOTRAN experiment. Papers will be submitted to recognized and appropriate scientific journals. Materials will also be prepared for presentation at the final IML-1 IWG meeting.

In the longer term we foresee the desirability of a number of follow up investigations as a direct result of the data obtained from the FOTRAN experiment. The dose response curve data did not provide sufficient data points to substantiate the impression that, under weightless conditions, the phototropic System I peak response shifted to higher dose levels. The FOTRAN experiment provided a broad range of stimulus doses to provide an overall picture of responses from Systems I, II and III.

It would now be appropriate to obtain a more detailed picture of the dose range over which the FOTRAN experiment found significant changes between the flight and ground data. Such an experiment could be essentially a re-flight of the FOTRAN experiment with changed stimulus schedules to cover the 3 - 99 second range in greater detail.

The nastic responses found during the FOTRAN experiment were probably a cause of some of the variability seen in the raw data. The fact that the seedling plane chosen for the phototropic response coincided with the natural plane of the nastic movement contributed to problems of interpretation of the responses. In any re-flight we would seriously consider moving the plane of the prime experimental response (phototropism) to one at right angles to the nastic response plane, thereby minimizing the interactions of the two response systems. This would be simply accomplished by selecting a different seed planting orientation.

Apart from their role as a interfering factor in the determination of phototropic and other responses, the nastic curvatures that developed in young seedlings are of interest in their own right. It could be of importance to future space flight investigations and/or crop growth as mentioned above. It would be of considerable interest to define more closely the conditions under which such nastic curvatures develop. Can the curvatures be prevented by, for example, using a light source as a directional cue during early growth? Relatively simple experimental equipment and protocols would provide the answers to such questions, but access to true microgravity (in orbit) would be required to obtain unequivocal answers. Clinostat experiments could provide preliminary information, but, as reinforced by the FOTRAN flight data, do not provide a perfect simulation of true microgravity. A flight investigation of the nastic responses of cereal seedlings and possible countermeasures could determine the importance of this phenomenon for future orbital crop production facilities.

The precocious development observed in both wheat and oat seedlings during the IML-1 mission can not be explained with the data available. Our apparent inability to predict the rate of seedling development in space on the basis of extensive ground studies is a matter of some importance in considering the timeline planning of future

experiments. The factor or factors responsible for the rapid development are apparently related to the environment within the GPPF and Spacelab environment. Microgravity and launch stresses can be ruled out as causative factors. At present, we believe that this is the first report of a germination enhancement. It would be important to know how widespread this type of effect might be. Can the effect be repeated for other species? Is the effect linked to some peculiarity of the GPPF equipment, or does it occur in wheat and oats in other situations during orbital flight? It would be of both practical and theoretical interest to identify the factor(s) responsible, an endeavor which would, of necessity, include a flight investigation. The scope of a suitable flight experiment could vary from a relatively simple middeck investigation to more ambitious projects suitable for either Spacelab or Space Station.

6.0 PUBLICATION LIST

The following is a list of publications directly associated with the FOTRAN experiment.

- 1973 Johnsson, A. & Heathcote, D.G.: Experimental evidence and models on circumnutation. *Z. Pflanzenphysiol.*, **70**, 371-405.
- 1977 Heathcote, D.G.: Circumnutation and gravity. *Proceedings of the European Symposium on Life Science Research in Space*. Cologne/Portz ESA SP 130, 195-201.
- 1981 Heathcote, D.G.: The geotropic reaction and statolith movements following geo-stimulation of mung bean hypocotyls. *Plant Cell & Environment*, **4**, 131-140.
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- 1987 Heathcote, D.G.: The role of nodal and internodal responses in gravitropism and autotropism in *Gallium aparine* L. *Plant Cell & Environment*, **10**, 701-703.
- 1987 Chapman, D.K., Heathcote, D.G. and Brown, A.H.: Light output from tungsten filament lamps during low gravity exposure on KC-135 parabolic flights. *ASGSB Bulletin*, **1**, 37.
- 1988 Heathcote, D.G. & Chapman, D.K.: Comparison of phototropic responses of wheat coleoptiles in flight hardware and clinostat tests. *ASGSB Bulletin*, **2**, 46.
- 1990 Heathcote, D.G., Brown, A.H. and Chapman, D.K.: FOTRAN: An experiment to investigate the effects of phototropic stimulations on the growth movements of wheat seedlings using the Gravitational Plant Physiology Facility on the IML-1 Spacelab Mission. *ASGSB Bulletin*, **4**, 56.
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- 1992 Brown, A.H., Chapman, D.K. and Heathcote, D.G.: Characterization of precocious seedling development observed during IML-1 mission. *ASGSB Bulletin*, **6**, 58.
- 1992 Chapman, D.K., Heathcote, D.G., Brown, A.H. and Johnsson, A.C.G.: Detection of apparent autotropic responses of seedlings under microgravity conditions on IML-1. *ASGSB Bulletin*, **6**, 59.
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- 1992 Brown, A.H., Chapman, D.K., Heathcote, D.G. and Johnsson, A.C.G.: Plant's tropistic responses in weightlessness. *29th Plenary Meeting COSPAR (World Space Congress)* p 530.
- 1993 Submitted to *Plant Cell & Environment* : Johnsson, A.C.G., Chapman, D.K., Brown, A.H., Johnson-Glebe, C., Karlsson, C and Heathcote, D.G.: Scatter in growth direction of plants under different g-values in the range of 1g to 20g.

7.0 ACKNOWLEDGEMENTS

The success of a project of this complexity is dependent on the efforts of a large number of individuals, including many that we, the scientific team, may not even have met, or even have been aware of their existence. With such a large number of people actively involved in ensuring the possibility of success for the FOTRAN experiment, it is somewhat invidious to mention specific individuals. However, major roles have been played by the following persons or groups, and it is fitting that their contribution is noted with gratitude.

The IML-1 Crew:	Ronald Grabe, Steven Oswald, William Reddy, Norman Thagard, David Hilmers, Roberta Bondar, Ulf Merbold, Roger Crouch, Ken Money
Ames Research Center:	Ron Ballard, Paul Callahan, Justina Grove, Tony Intravaia, Joellen Lashbrook, Larry Lenke, Rich McKenna, Chuck Winget, Gary Plapp, Ron Schaefer, Ken Souza
Marshall Space Flight Center:	Fred Applegate, John Frazier, Kent Lasley, Robert McBrayer, Tina Melton, Teresa Miller, Kathy Nordman, Julie Sanchez, Robert Snyder, POCC Cadre Members.
Kennedy Space Center:	Bill Knot, Bill Muncey, Damon Nelson, Mimi Shao, Hangar L Staff.
GPPL, Philadelphia:	Na Chen, Corey Johnson, Bob Lewis, Brian Oldratti, Maria Rachko, Allen Venditti, Dick Wallace
AVH, Trondheim, Norway:	Terje Eidesmo, Anders Johnsson, Christina Karlsson, Eli Zacharriassen
UCC Cardiff, U.K.:	Bryn Bircher